NPC Natural Product Communications

2018
Vol. 13
No. 7
891 - 893

Volatiles of *Tanacetum macrophyllum* Obtained by Different Extraction Methods

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Received: March 22nd, 2018; Accepted: April 18th, 2018

Terpene compounds of *Tanacetum macrophyllum* as 1) essential oils, obtained by hydrodistillation (HD), 2) essential oil extracts, obtained by simultaneous hydrodistillation and extraction (SDE) and 3) volatiles, obtained by Static Headspace GC-MS analysis (HS) were processed. Monoterpenes were the most dominant (49.2%, 49.5% and 90.4%, respectively). Profiles of essential oils obtained by HD and SD were quite similar, with oxygenated monoterpenes (39.3% and 39.4%) being the most abundant. In HS volatiles oxygenated monoterpenes also dominated (57.4%). Total sesquiterpenes were abundant in HD and SDE volatiles (38.2% and 39.2%, resp.), where sesquiterpene hydrocarbons were prevalent (27.3% and 28.7%, resp.). Germacrene D was dominant in HD and SDE oils (22.0% and 23.3%, resp.) and 1,8-cineole in HS volatiles (34.3%). To our knowledge, this is the first use of Headspace technique on *T. macrophyllum*. Furthermore, this is the first comparison of different techniques of volatile extraction in *T. macrophyllum*.

Keywords: *Tanacetum macrophyllum*, Large-leafed tancy, Volatiles, Hydrodistillation, Hydrodistillation and extraction, Headspace.

Large-leafed tancy (*Tanacetum macrophyllum* (Waldst. et Kit.) Sch. Bip. belongs to family Asteraceae. It is morphologically similar with *Achillea grandifolia* [1] which could make some risks in folk medicine. It shows antimicrobial, anticoagulant and antifibrinolytic activities ([2] and refs. cited therein). Comparing different techniques of obtaining essential oils, extracts and volatiles in plant species were used very often [3–6], etc. HS-GC/MS method is considered to be useful for fast chemical screening of volatiles in foods and plants [4]. The aims of this work are to investigate chemical composition of *T. macrophyllum* wild-growing on high elevation in south-west Serbia as well as to compare the effects of different techniques on composition and profiles of its volatile compounds. To our knowledge, this is the first use of Headspace technique on *T. macrophyllum*.

Terpene compounds of *Tanacetum macrophyllum* 1) essential oils, obtained by hydrodistillation (HD), 2) extracts, obtained by simultaneous hydrodistillation and extraction (SDE) and 3) aroma volatiles, obtained by Headspace GC-MS analysis (HS) were summarized in Table 1. A total number of 58, 55, and 31 compounds out of 82, 71, and 40 of *T. macrophyllum* were identified by HD, SDE and HS, resp., comprising 62 identified out of 91 detected compounds (Table 1). Sesquiterpene hydrocarbon, germacrene D, was the most abundant compound in HD and SDE oils (Figure 1). Two oxygenated monoterpenes, camphor and 1.8 cineole, and one oxygenated sesquiterpene, caryophyllene oxide, were also abundant regardless of the extraction technique. Only in HS volatiles, one monoterpene hydrocarbon, sabinene, was one of dominant terpenes.

Dominant terpenes of HD ess.oil of *T. macrophyllum* had following profile: *germacrene D >> camphor = 1.8 cineole >> camphene = caryophyllene oxide*. (the following symbols are used to denote differences in content: 0.1–1.0% (=); 1.1–5.0% (>); 5.1–15.0% (>>); more than 15.1% (>>>), after [7]. Together with additional twenty three compounds they presented higher amount of total monoterpenes (49.2%) (especially oxygenated ones: 39.3%) than total sesquiterpenes (38.2%) (Tables 1, 2). SDE extract of *T. macrophyllum* had identical profile of main compounds and approximately the same content. These compounds plus twenty six additional ones presented higher amount of total monoterpenes (49.5%) (especially oxygenated: 39.4%) than total sesquiterpenes (39.2%). HD and SDE terpene profiles of *T. macrophyllum* were nearly identical in contents of almost all compounds, but tricosane was found only in HD ess. oil, while SDE extract had twice more *cis*-spiroether.

Profile of main terpene compounds of HS volatiles of *T. macrophyllum* was: *1,8-cineole >>> camphene > camphor > > sabinene = borneol*. This profile as well as overall terpene profile was extremely rich in monoterpenes (Table 1) (Figure 1), owing to 2–6 times more abundant tricyclene, α-thujene, α-pinene, etc., which leaded to strong domination of total monoterpenes (90.4%) over total sesquiterpenes (6.2%) (Table 2), especially higher oxygenated monoterpenes over monoterpene hydrocarbons (57.9% and 26.9%, respectively). Three compounds were found only in HS volatiles.

To the best of our knowledge, until now, ca. 124 compounds of *T. macrophyllum* essential oil, obtained by hydrodistillation, were identified in Serbia ([1,8] and presented results). Large-leafed tancy from SW Serbia, with dominant germacrene D (Table 1) is quite different from two other Serbian ones where: isobornyl acetate + borneol + 1,8-cineole (SE Serbia, Mt. Suva planina, [1]) and

Table 1: Terpene compounds (in %) in the aerial parts of *T. macrophyllum*

Entry	RT[a)]	RI[b)]	Compds	HD	SDE	HS	Class
1	3.740	829	C_9H_{14}	0.9	0.9	-	CC
2	3.895	839	C_9H_{14}	0.1	tr	-	CC
3	5.470	921	Tricyclene	0.2	0.2	1.0	MH
4	5.568	924	*α*-Thujene	0.2	0.1	1.2	MH
5	5.749	931	*α*-Pinene	**1.6**	**1.8**	**3.3**	**MH**
6	6.143	945	**Camphene**	**3.9**	**4.1**	**17.9**	**MH**
7	6.811	970	**Sabinene**	**2.2**	**2.3**	**4.4**	**MH**
8	6.921	974	*β*-Pinene	0.9	0.7	0.7	MH
9	7.340	989	2,3-Dehydro-1,8-cineole	0.3	0.3	tr	OM
10	7.412	992	1,3,5-Trimethylbenzene	-	-	0.2	BC
11	8.158	1014	*α*-Terpinene	0.1	0.1	0.1	MH
12	8.355	1020	1,2,4-Trimethylbenzene	0.2	-	-	BC
13	8.419	1021	*p*-Cymene	0.2	0.2	0.7	MH
14	8.576	1026	Limonene	0.4	0.4	2.5	MH
15	8.653	1028	**1,8-Cineole**	**15.7**	**16.0**	**34.3**	**OM**
16	9.580	1054	Unknown 1	-	-	0.3	-
17	9.640	1055	*γ*-Terpinene	0.2	0.2	0.9	MH
18	9.935	1063	(*Z*)-Sabinene hydrate	0.3	0.4	-	OM
19	10.782	1087	Terpinolene	tr	tr	tr	MH
20	11.131	1096	(*E*)-Sabinene hydrate	0.3	0.3	0.4	OM
21	11.193	1098	Linalool	0.1	0.1	0.4	OM
22	11.377	1102	Nonanal	0.1	tr	-	AC
23	12.107	1119	*trans*-*p*-Menta-2,8-dien-1-ol	0.2	0.2	-	OM
24	12.212	1122	*Chrysanthenone*	-	-	1.1	OM
25	12.911	1135	*trans*-Pinocarveol	0.2	0.2	-	OM
26	13.031	1140	**Camphor**	**16.4**	**17.0**	**16.3**	**OM**
27	13.788	1158	*cis-Chrysanthenol*	1.2	1.1	-	OM
28	13.919	1161	**Borneol**	**2.2**	**1.9**	**3.5**	OM
29	14.429	1172	Terpinene-4-ol	0.6	0.4	-	OM
30	14.430	1172	Unknown 2	-	-	0.2	-
31	15.007	1186	*α*-Terpineol	0.8	0.6	-	OM
32	15.007	1186	Unknown 3	-	-	0.6	-
33	18.128	1257	*cis*-Chrysanthenyl acetate	0.1	0.1	tr	OM
34	19.211	1281	Bornyl acetate	tr	tr	1.0	OM
35	22.048	1346	*α*-Terpinyl acetate	-	-	-	OM
36	22.790	1362	Cyclosativene	0.5	0.5	0.9	SH
37	23.220	1373	*α*-Copaene	1.3	1.3	2.1	SH
38	23.608	1381	*β*-Bourbonene	0.1	tr	0.1	SH
39	23.724	1383	Unknown 4	tr	-	0.1	-
40	24.508	1402	Unknown 5	0.2	0.2	0.1	-
41	24.909	1411	Unknown 6	0.1	0.1	-	-
42	25.105	1416	(*E*)-Caryophyllene	1.1	1.2	0.3	SH
43	25.119	1416	Unknown 7	-	-	1.0	-
44	25.522	1426	*β*-Copaene	0.1	0.1	-	SH
45	26.562	1452	*α*-Humulene	0.2	0.2	tr	SH
46	26.797	1458	(*E*)-*β*-Farnesene	-	-	0.2	SH
47	26.999	1458	Allo-Aromadendrene	0.2	0.3	tr	SH
48	27.598	1470	Unknown 8	0.1	-	-	-
49	27.808	1483	**Germacrene D**	**22.0**	**23.3**	**1.9**	**SH**
50	27.980	1485	Unknown 9	0.3	0.2	1.0	-
51	28.099	1488	Unknown 10	0.4	0.3	tr	-
52	28.424	1495	Bicyclogermacrene	1.2	1.2	-	SH
53	28.828	1504	Bornyl isovalerate	0.4	0.4	0.4	OM
54	28.909	1507	(*E,E*)-*α*-Farnesene	0.4	0.3	-	SH
55	29.114	1512	Unknown 11	-	-	0.2	-
56	29.226	1514	Isobornyl isovalerate	0.5	0.4	-	OM
57	29.526	1521	*δ*-Cadinene	0.2	0.3	-	SH
58	30.819	1552	Unknown 12	0.1	-	-	-
59	31.317	1564	Unknown 13	0.3	0.4	-	-
60	31.669	1572	Unknown 14	0.8	0.8	-	-
61	31.763	1576	Spathulenol	1.1	0.8	-	OS
62	31.860	1578	Unknown 15	0.8	0.4	-	-
63	31.990	1581	**Caryophyllene oxide**	**3.7**	**3.5**	**0.7**	**OS**
64	32.169	1584	Unknown 16	0.1	-	-	-
65	32.249	1588	*β*-Copaene-4-*α*-ol	0.5	0.6	-	OS
66	32.386	1591	Salvial-4(14)-en-1-one	0.5	0.6	-	OS
67	32.677	1598	Unknown 17	0.1	-	-	-
68	32.833	1602	Unknown 18	1.5	1.5	-	-
69	33.008	1605	Unknown 19	0.2	0.2	-	-
70	33.139	1609	Unknown 20	0.2	0.2	-	-
71	33.345	1615	Junenol	0.1	0.1	-	OS
72	33.580	1620	Unknown 21	0.1	-	-	-
73	33.752	1625	Muurola-4,10(14)-dien-1-*β*-ol	0.6	0.6	-	OS
74	33.904	1629	*γ*-Eudesmol	0.5	0.4	-	OS
75	34.071	1633	Caryophylladienol I	1.3	1.3	-	OS
76	34.450	1644	Unknown 22	0.2	0.2	-	-
77	34.719	1651	Unknown 23	0.6	0.4	-	-
78	35.189	1663	Unknown 24	0.2	tr	-	-
79	35.407	1668	14-Hydroxy-9-epi-(*E*)-caryophyllene	0.2	0.2	-	OS
80	35.699	1676	Mustakone	0.7	0.7	-	OS
81	36.012	1684	Germacra-4(15),5,10(14)-triene-1-*α*-ol	1.5	1.5	-	OS
82	38.562	1754	Unknown 25	0.7	-	-	-
83	38.888	1763	Unknown 26	0.7	0.6	-	-
84	39.121	1796	14-Oxy-*α*-muurolene	0.2	0.2	-	OS
85	41.420	1834	Unknown 27	0.7	0.7	-	-
86	42.947	1878	*cis*-Spiroether	1.3	2.5	-	OT
87	43.482	1891	*trans*-Spiroether	0.8	0.8	-	OT
88	50.131	2099	Unknown 28	0.2	-	-	-
89	50.213	2101	Unknown 29	0.5	0.9	-	-
90	56.232	2305	Tricosane	0.1	-	-	AC
91	61.828	2502	Pentacosane	tr	-	-	AC
		Total	**No. of compounds**	**82**	**71**	**40**	
		Total	**No. of identified compounds**	**58**	**55**	**31**	

[a)]RT – retention time; [b)]RT – retention index.

Table 2: Terpene classes (in %) in the aerial parts of *T. macrophyllum*

Class	HD	SDE	HS
Total monoterpenes	**49.2**	**49.5**	**90.4**
Monoterpene hydrocarbons (MH)	9.9	10.1	33.0
Oxygenated Monoterpenes (OM)	39.3	39.4	57.4
Total sesquiterpenes	**38.2**	**39.2**	**6.2**
Sesquiterpene hydrocarbons (SH)	27.3	28.7	5.5
Oxygenated sesquiterpenes (OS)	10.9	10.5	0.7
Others	**3.5**	**4.2**	**0.2**
Aliphatic compounds (AC) [a)]	0.2	0.0	0.0
Cyclic compounds (CC) [b)]	1.0	0.9	0.0
Benzenoid compounds (BC) [b)]	0.2	0.0	0.2
Others (OT)	2.1	3.3	0.0
Unknown	**9.1**	**7.1**	**3.2**
Total (%)	**100.0**	**100.0**	**100.0**

a) Aliphatic aldehydes and hydrocarbons; [b)] Cyclic hydrocarbons;

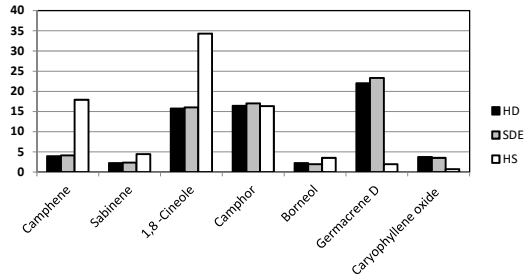

Figure 1: Profile of main terpene compounds of *T.macrophyllum*

γ-cadinene (south Serbia, Mt. Šara, [8]) strongly dominated. They are quite different from tancies from Turkey with dominant *β*-eudesmol [9, 10] and *γ*-eudesmol+copaborneol [11]. These populations are richer in some oxygenated sesquiterpenes (especially in eudesmanes and *E*-sesquilavandulol) and oxygenated monoterpenes: *β*-thujone, bornyl acetate and *cis*-chrysanthenol. Large-leafed tancy with dominant *p*-methyl benzyl alcochol [12] and abundant *δ*-cadinene, differs from listed terpene profiles. Population from SW Serbia also has the highest values of camphor and 1,8-cineole among all investigated populations of *T. macrophyllum*. Furthermore, some authors regarded that essential oils of this species belonged to sesquiterpenoid-type group ([13] and refs. cited therein). In present results the most abundant was germacrene D (22%), but total monoterpenes dominated (49.2%).

This is the first use of Headspace technique on *T. macrophyllum*. Furthermore, this is the first comparison of different techniques of volatiles extraction in *T. macrophyllum*.

Experimental

Plant material: Fresh herbs were collected in September 2015 from South-West Serbia, Mt. Zlatar, Gradina locality near the village Pravoševo (43°22'53", 19°45'52", elevation: 1320–1427 m). Natural habitats of this species was described earlier [14].

Extraction and isolation: Three different methods of essential oil extraction from air dried above-ground parts of plants were used: 1) Hydrodistillation via Clevenger apparatus, 2) Simultaneous distillation and extraction with dichloromethane via Likens-

Nickerson apparatus, and 3) Extraction via Static Headspace sampling apparatus.

GC-FID and GC/MS analyses: GC-FID and GC/MS analyses were carried out with an Agilent 7890A apparatus equipped with an 5975C MSD, FID, and a HP-5MSI fused-silica cap. col. 30 m × 0.25 mm × 0.25 μm). For HS analyses 2000 μL of generated vapor was drawn out from the vial and injected directly into the gas chromatograph using a heated gas-tight syringe (105°C). The oven temp. was programmed linearly rising from 60 to 315 °C for 15 min; injector: 250°C; FID detect.: 300°C; carrier gas, He (1.0 mL/min at 210°C), injection vol. 1 μL (for CL and LN) or 2 mL (for HS), split ratio, 10:1. EI-MS (70 eV), m/z range 40–550.

Compound identification: Identification of all compounds in analyses was match by comparison of their linear retention indices (relative to C8–C36 *n*-alkanes on the HP-5MSI column) and MS spectra with those of authentic standards from NIST11 and homemade MS library data bases.

Acknowledgements – This research was supported by Grants Nos 173029, 173021 and 172053 by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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